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RADIATION AND HEAT RESISTANCE OF MORAXELLA-ACINETOBACTER IN MEA--ETC(U)
JAN 78 R B MAXCY, D B ROWLEY, A ANELLIS DAAG17-76-C-0008
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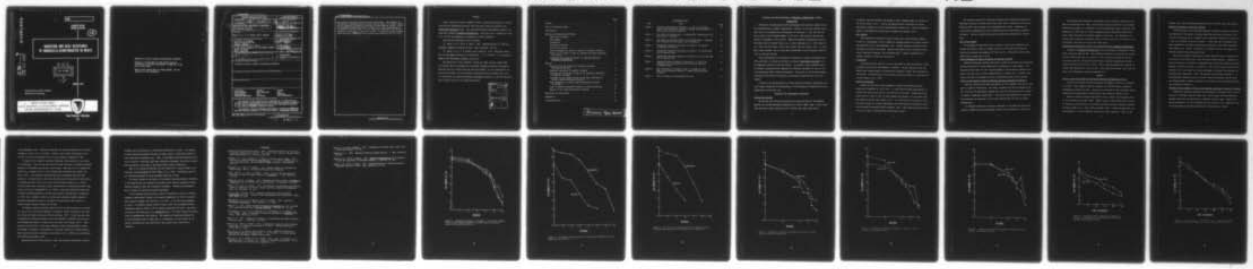

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RADIATION AND HEAT RESISTANCE OF MORAXELLA-ACINETOBACTER IN MEATS

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The purpose of this research was to determine: (1) if fresh pork and chicken contained asporogenous bacteria which were more radiation resistant at -30°C than C. botulinum spores; and (2) factors affecting the survival of such radiation-resistant bacteria in meats.

Asporogenous bacteria belonging to the genera Moraxella-Acinetobacter, which are more radiation resistant at -30°C than spores of Clostridium botulinum,

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were isolated from minced fresh pork and chicken wings. The frequency of occurrence of resistant cells was 10-100 cells per gram. Fat content (5-44%) did not influence the radiation resistance of these bacteria in meat. Radiation resistant isolates were unable to multiply in either vacuum-packed or air-packed minced beef because of their high water requirement (A_w) and were sensitive to heat (D_{68^oC} in beef was 9.3 min). The shoulder of the radiation death curve was eliminated if broth cultures were heated at 70°C for 5 min.

D sub 68 C

PREFACE

Under a previous contract (DAAK03-74-0072) a scheme was developed to isolate from raw beef asporogenous bacteria that were more radiation resistant than Clostridium botulinum spores. The radiation-resistant asporogenous bacteria isolated were primarily Moraxella-Acinetobacter. They grew over a temperature range of 2° to 50°C and appeared to be sensitive to heat treatments.

This work was published in the following:

(1) Welch, A. B., and R. B. Maxcy. 1975. Characterization of radiation-resistant vegetative bacteria in beef. Appl. Microbiol. 30: 242.

(2) Maxcy, R. B., D. B. Rowley, and A. Anellis. 1976. Radiation resistance of asporogenous bacteria in frozen beef. T. R. 76-43-FSL. U.S. Army Natick Research and Development Command, Natick, MA.

The objectives of this research, carried out under contract number DAAG 17-76-C-008, were to determine (1) if similar radiation-resistant asporogenous bacteria could be isolated from raw pork and chicken, (2) the concentration of such cells in meats, (3) the radiation resistance of heat stressed cells, and (4) the ability of radiation-resistant asporogenous bacteria to survive and multiply in either vacuum or air packed minced meat.

ILLUSTRATIVE DATA

RADIATION AND HEAT RESISTANCE OF MORAXELLA-ACINETOBACTER IN MEATS

INTRODUCTION

Irradiation processing may provide prepackaged food stability against microbial decomposition at ambient temperatures. However, the process requires assurance that all contaminating microorganisms are destroyed, or that they are not able to grow in the microenvironment of the food. Some specific bacteria may provide a challenge to this process. Radiation-resistant asporogenous bacteria have been found in various foods, e.g., in beef (Anderson et al., 1956; Maxcy et al., 1976), in fish (Lewis, 1973), and in grain (Ito and Iizuke, 1971). A systematic study, however, has not been made to determine if such resistant bacteria occur in pork and chicken.

This investigation was undertaken to determine if similar asporogenous bacteria resistant to radiation sufficient to destroy Clostridium botulinum occur in commercial sources of pork and chicken. In addition, these organisms were to be studied to determine their magnitude of radiation resistance as well as factors influencing their radiation resistance. The nature of the microenvironment influencing the radiation resistance and subsequent outgrowth were to be considered.

A study of the characteristics of these radiation-resistant asporogenous bacteria should contribute to understanding of the mechanism of resistance and their significance in food spoilage.

MATERIALS AND EXPERIMENTAL PROCEDURES

Plating and counting

The plating and counting procedures were those described by "Recommended Methods for the Microbiological Examination of Foods" (APHA, 1966). Plate counts were made after aerobic growth at 32°C on Plate Count Agar (PCA; Difco).

Incubation time was extended long enough to assure maximum number of colonies on the plates (Maxcy, 1977). Culture and identification techniques were those described in "Manual of Clinical Microbiology" (Tatum et al., 1974) and "Bergey's Manual of Determinative Bacteriology" (Buchanan and Gibbons, 1974).

Meat samples

Pork samples were obtained as minced fresh pork, which had been prepared locally in a supermarket, and as commercial packages prepared in central processing operations from a wide geographic area. Chicken samples consisted of skin, cartilage, and muscle from wings. Samples represented locally processed birds, central packing in Arkansas, and in Massachusetts. Samples represented the seasonal extremes of warm and cold weather.

Irradiation

A Cobalt-60 source similar to the one described by Teeny and Miyauchi (1970) provided a dose rate of approximately 8 krad/min. Unless otherwise noted, the samples were vacuum packed at 125-mm mercury pressure, frozen, and irradiated at -30° ± 10°C. Further details on the procedure have been given in a previous publication (Maxcy et al., 1976).

Isolation procedures

Samples of meat were vacuum packaged in flexible polyethylene pouches, frozen, and irradiated at -30° ± 10°C. A 1 Mrad dose provided approximately 10 colonies per plate on PCA after a 1:10 dilution of the meat. Incubation of the plates was for 5 days at 32°C to allow adequate opportunity for the slower growing radiation injured cells (Maxcy, 1977). All colonies up to as many as 10 per plate were picked for further observations and classification according to previously described procedures (Welch and Maxcy, 1975). When there were more than 10 colonies per plate, representative colonies were chosen.

The selective process for radiation resistance was continued by growing the individual isolates in m-Plate Count Broth (PCB; Difco), freezing in the culture medium in which they had grown, and irradiating with 2 Mrad. Comparison of counts before irradiation and after irradiation gave an indication of relative resistance. The most radiation-resistant cultures were maintained for further study.

Pure culture growth

For storage and inoculation, pure cultures of the various isolates to be studied were grown in PCB on a shaker incubator at 32°C until the broth was turbid and contained approximately 10^9 cells per ml. Storage for culture maintenance after growth was at 3-5°C.

Vacuum packaging and impact on growth of resistant isolates

Radiation-resistant isolates were grown in PCB and inoculated into previously radiation-sterilized (2 Mrad) minced meat by grinding in a food chopper. The inoculated minced meat was then vacuum packaged at 125-mm mercury pressure with aseptic care and set at various temperatures for storage tests. Periodic plate counts were made to determine the fate of the bacteria used as the inoculum.

Effect of fat content of meat on radiation and heat resistance of bacteria

Specially selected lean beef containing 3.0-7.0% (average 5.2%) fat was compared to high-fat minced beef. The latter contained 42.0-46.5% (average 44.4%) fat, which was the approximate maximum that could be incorporated into a product to simulate "commercial ground beef." The inocula were dispersed in radiation-sterilized meat as described in the section dealing with the fate in vacuum-packaged meat.

To determine comparative radiation resistance in the high-fat and low-fat products, samples were vacuum packaged, frozen, and irradiated at -30° ± 10°C.

To determine heat resistance, the method of Welch and Maxcy (1975) was used. Meat was irradiated with 2 Mrad, then inoculated with the bacteria to be studied. Samples were pressed to a thickness of 3 mm in polyethylene bags. They were then immersed in a water bath for various temperature-time combinations after which plate counts were made to determine numbers of survivors. The temperature for heating each culture was ultimately chosen to obtain destruction of bacteria over sufficiently long time to allow proper time control.

Estimating the population density of radiation-resistant Moraxella-Acinetobacter

Estimates of Moraxella-Acinetobacter (M-A) were based on differential counts. Total counts were made on PCA. The radiation-resistant count was made by plating on PCA, allowing the agar to solidify at 32°C, followed by pouring an overlay. The plates were then irradiated with 400 krad at ambient temperature and subsequently incubated for 48-72 hr at 32°C. Spore counts were made after heating the initial test material for 10 min at 80°C. The radiation-resistant count less the spore count represented radiation-resistant M-A.

RESULTS

Survey of pork and chicken for radiation-resistant asporogenous bacteria

Survey for resistant isolates was made on 10 samples of pork and 10 samples of chicken. These samples commonly contained from 10-100 radiation-resistant asporogenous bacteria per gram. From the total isolates 36 with different morphological and/or physiological characteristics were taken for further study.

When the various isolates were grown in broth, frozen, and irradiated only 3 of the 36 isolates survived 2 Mrad. Further study of these showed them to be M-A of identical characteristics to the highly radiation-resistant bacteria isolated from beef (Maxcy et al., 1976; Welch and Maxcy, 1975). These had been given Isolate numbers 4, 7, and 13 based on the order of their isolation. Thus, it was

apparent that highly radiation-resistant M-A occur in beef, pork, and chicken.

Radiation resistance of specific isolates

Further studies of the radiation-resistant isolates were made to determine the magnitude of resistance in various media with particular attention to the pattern of the death curve. Examples of the radiation resistance of Isolates 4, 7, and 13 in chicken are given in Figure 1. These data are in agreement with those reported for the same isolates when irradiated in beef (Welch and Maxcy, 1975; Maxcy et al., 1976). A shoulder in the death curve is also apparent as had been observed when working with beef.

The extreme radiation resistance of these vegetative cells is apparent in the pattern of the death curve, which involves a major shoulder. Combined destructive factors as proposed for radappertizing of meat were therefore studied. Heating a culture of Isolate 4 in broth for 5 min at 70°C reduced the population between 1 and 2 log cycles, and during subsequent irradiation the reduction in population was logarithmic. Thus, the shoulder was eliminated (Figure 2). A less radiation-resistant culture, Isolate 9 which was obtained from both beef and chicken showed a similar alteration in the death curve by combining heating and irradiation (Figure 3).

Influence of fat content of beef on the radiation resistance of specific isolates

Comparative radiation resistance was determined in extremely low-fat minced beef and in extremely high-fat minced beef. Three highly radiation-resistant isolates were used with duplicate or triplicate trials on each. Results presented in Figures 4, 5, and 6 indicate the fat content is not a significant factor influencing the radiation resistance of these bacteria in meat.

Influence of fat content of beef on the heat resistance of certain radiation-resistant bacteria

Low-fat minced beef and high-fat minced beef as previously described were used to suspend certain highly radiation-resistant isolates for observations on heat resistance. Temperatures of heating were chosen to obtain a gradual destruction of bacteria for accurate control of time and to get enough destruction to allow calculation of the D_{10} value. The results for duplicate trials with Isolates 4, 7, and 13 were averaged and presented in Figure 7. At 68°C the D_{10} values for low-fat and high-fat meat were 9.2 and 9.3 min, respectively, thus indicating the fat content was without effect in these experiments.

Heat resistance of certain radiation-resistant isolates in chicken

At 72°C the D_{10} value for Isolate 4 was found to be 6.6 min and for Isolate 7 it was 7.3 min (Figure 8). These values were in general agreement with data on beef as reported above and in a previous publication (Welch and Maxcy, 1975).

Fate of radiation-resistant isolates in vacuum packed meat

When isolates were mixed in minced beef or chicken, vacuum packaged at 125-mm mercury, and incubated at 32°C, there was little, if any, change in numbers. Data given in Figure 9 exemplify the results. Temperatures of 5, 10, 20, 27, and 32°C were tried for up to 18 days to see if there was growth. None grew significantly. These radiation-resistant isolates were not able to grow in either vacuum-packed or air-packed minced meat.

Failure of the radiation-resistant isolates to grow in meat was indeed unexpected since the origin was meat. Various approaches were taken to determine why these organisms failed to grow. The results indicated that the water activity (A_w) of fresh meat was below that required for growth of these organisms. As much as 10% water added and mixed with the meat was required to support

significant growth. A fuller proof of this concent will be given by data presented elsewhere.

Habitat of the radiation-resistant isolates

A great variety of samples was taken to represent different microenvironments. Normally, four samples were taken from each source indicated. The differential test for indications of radiation-resistant bacteria was run on each sample. Emphasis was given items that might have a vector to meat. Some areas of exploration were human hair, hands, animal hair, feathers, intestinal contents, soil of livestock pens, animal feed, sewage, and farm soil. Radiation-resistant M-A cells were isolated from each of these sources. With exception of samples from humans, the total counts were over 10 million per gram. The radiation-resistant M-A count was from 0.01 to 1% of the total count. One of the consistently rich sources of radiation-resistant M-A was hair from beef animals. Poultry feathers and litter were nearly as rich a source. The most surprising result was that radiation-resistant M-A were found in so many sources, and there was no apparent explanation in terms of commonly recognized microenvironmental factors.

DISCUSSION AND CONCLUSIONS

Radiation resistance of vegetative bacteria ranges from the extreme sensitivity exhibited by certain pseudomonads (Thornley, 1963; Maxcy and Tiwari, 1973; Green and Kaylor, 1977) to the extremely resistant micrococci (Anderson et al., 1956) and M-A (Welch and Maxcy, 1975). There is a continuum in relative resistance when the many types of bacteria are considered. Vegetative bacteria of intermediate to high resistance are widely scattered in nature. Furthermore, their occurrence is not predominantly associated with a particular season of the year, because the sampling program of this work included seasonal variation and

a wide geographic area. Radiation resistance was the same whether the cells were suspended in beef, pork, or chicken. Further, even extreme differences in fat content of beef had no apparent effect on the radiation resistance of M-A.

In light of the presently available knowledge, these bacteria do not appear to be important. They are not associated with food spoilage in presently accepted methods of processing, distribution, and storage. They only occur in significant numbers as a residual flora of low or medium dose irradiated food (Tiwari and Maxcy, 1972). The extremely radiation-resistant asporogenous bacteria were present at a concentration of less than 100 per gram as indicated by these results on chicken and pork, as well as by the work with beef by Maxcy et al. (1976). The only known study reporting a higher concentration of radiation-resistant vegetative cells was by Krabbenhoft et al. (1965). They were working with meat from a single processing operation, whereas the presently reported work, irradiating at -30°C, was a systematic effort to isolate and quantitate highly radiation-resistant asporogenous bacteria from meats collected from a wide variety of sources during different seasons of the year.

The highly radiation-resistant bacteria found in this work were not a product of a native flora having been exposed to radiation, thereby producing a few aberrant forms with higher resistance (Welch and Maxcy, 1975). On the contrary, when a population of radiation-resistant cells was exposed to radiation the survivors were weakened and unable to compete with the native flora (Maxcy, 1977). These results allay the fear of still more resistant strains being developed in meat processing, in contrast to development of increased resistance of other bacteria under special laboratory conditions (Licciardello et al., 1969; Corry and Roberts, 1970; Davies and Sinskey, 1973).

Characteristics of these bacteria, other than radiation resistance, perhaps

minimize their significance in irradiation preservation of foods. For example, the most radiation-resistant M-A were not able to grow in fresh meat because of their high water requirement (A_w). Thus, if the highly radiation-resistant cells were to survive a relatively high dose irradiation treatment, they would not grow when dispersed in fresh meat or any meat product without added water.

Many of the radiation-resistant M-A were sensitive to reduced oxygen occurring with vacuum packaging of foods (Maxcy et al., 1976). Furthermore, most of the radiation-resistant M-A were extremely sensitive to heat.

In further studies of the nature of the highly radiation-resistant isolates, it was observed that the shoulder of the death curve could be altered or eliminated by sequential heat and irradiation treatments. Perhaps this phenomenon has to do with the injury and recovery mechanism.

In the radappertization process, foods are formulated, placed in cellulose casings or metal molds, heated to an internal temperature of 73-77°C to inactivate autolytic enzymes, and chilled to -3 to 5°C. It is then vacuum packaged in cans or in flexible pouches, frozen to about -40°C, and irradiated within a temperature range of -40°C to -8°C to obtain the desired 12-D dose. The latter is based on the destruction of C. botulinum spores. No viable bacteria have been found in radappertized food products. The absence of radiation-resistant M-A in such processed foods is likely due to a combination of such factors as low initial concentration, heat sensitivity, heat injury, and a high dose of radiation.

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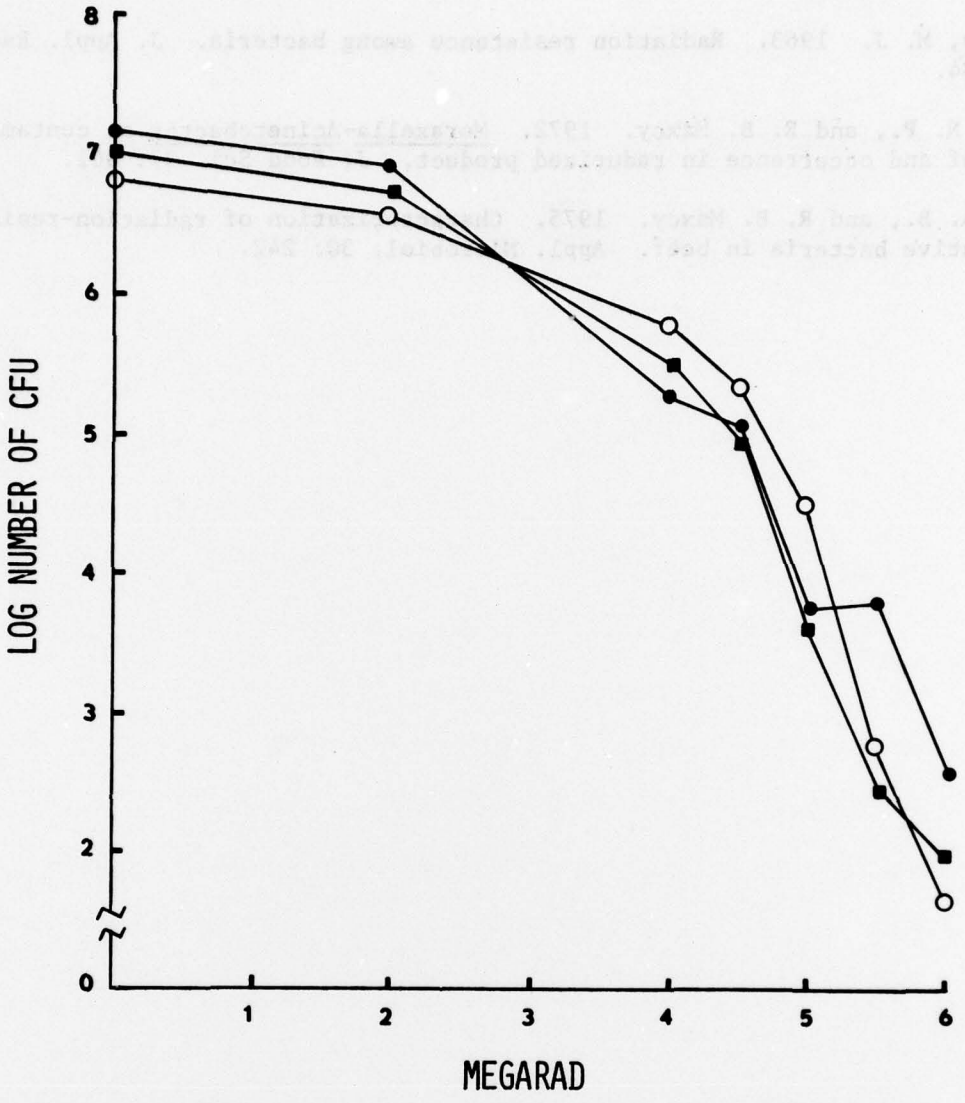

Figure 1. Radiation resistance of Isolates 4, 7 and 13 in chicken. Closed circles indicate Isolate 4; squares indicate Isolate 7; open circles indicate Isolate 13.

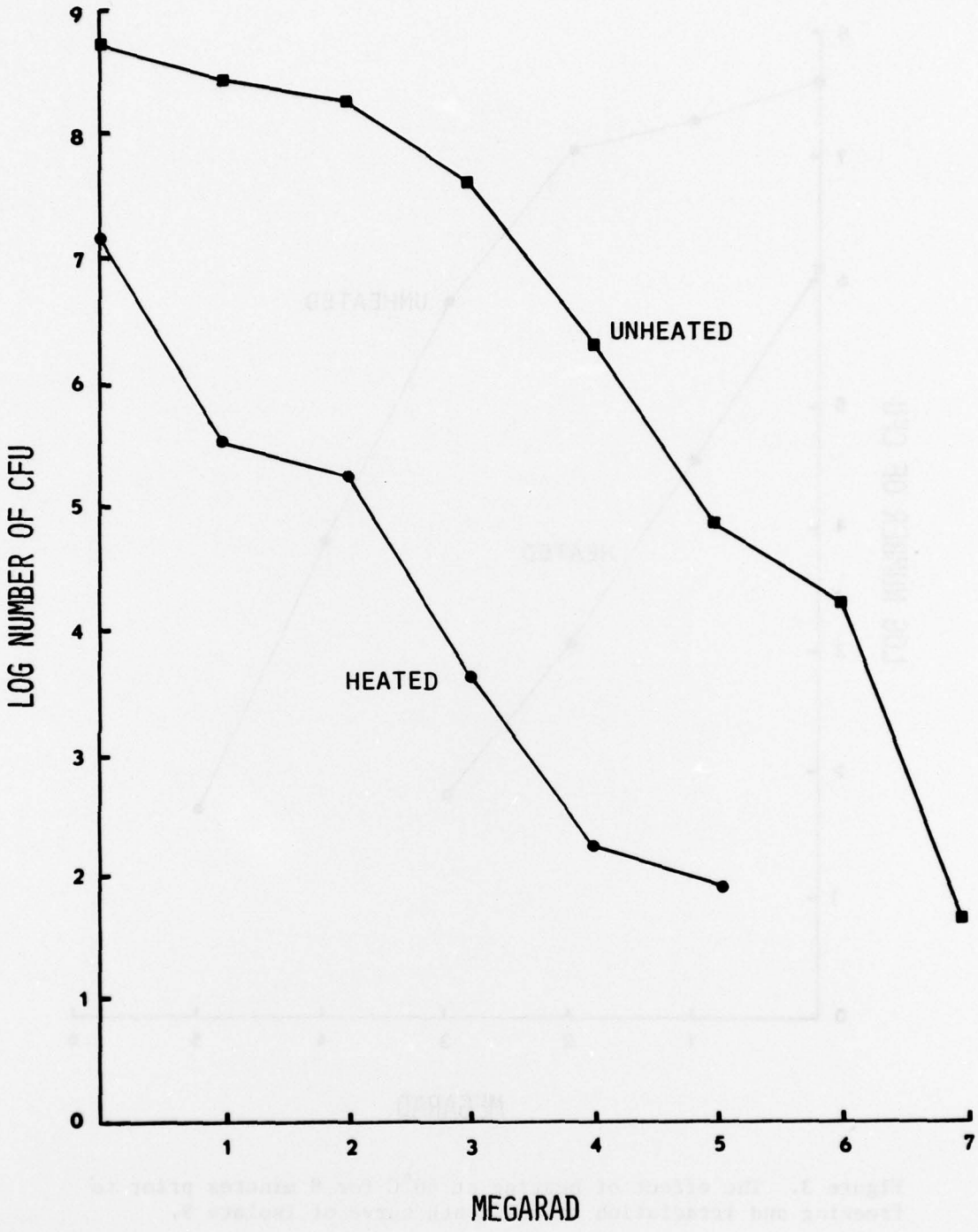

Figure 2. The effect of heating prior to freezing and irradiation on the death curve of Isolate 4.

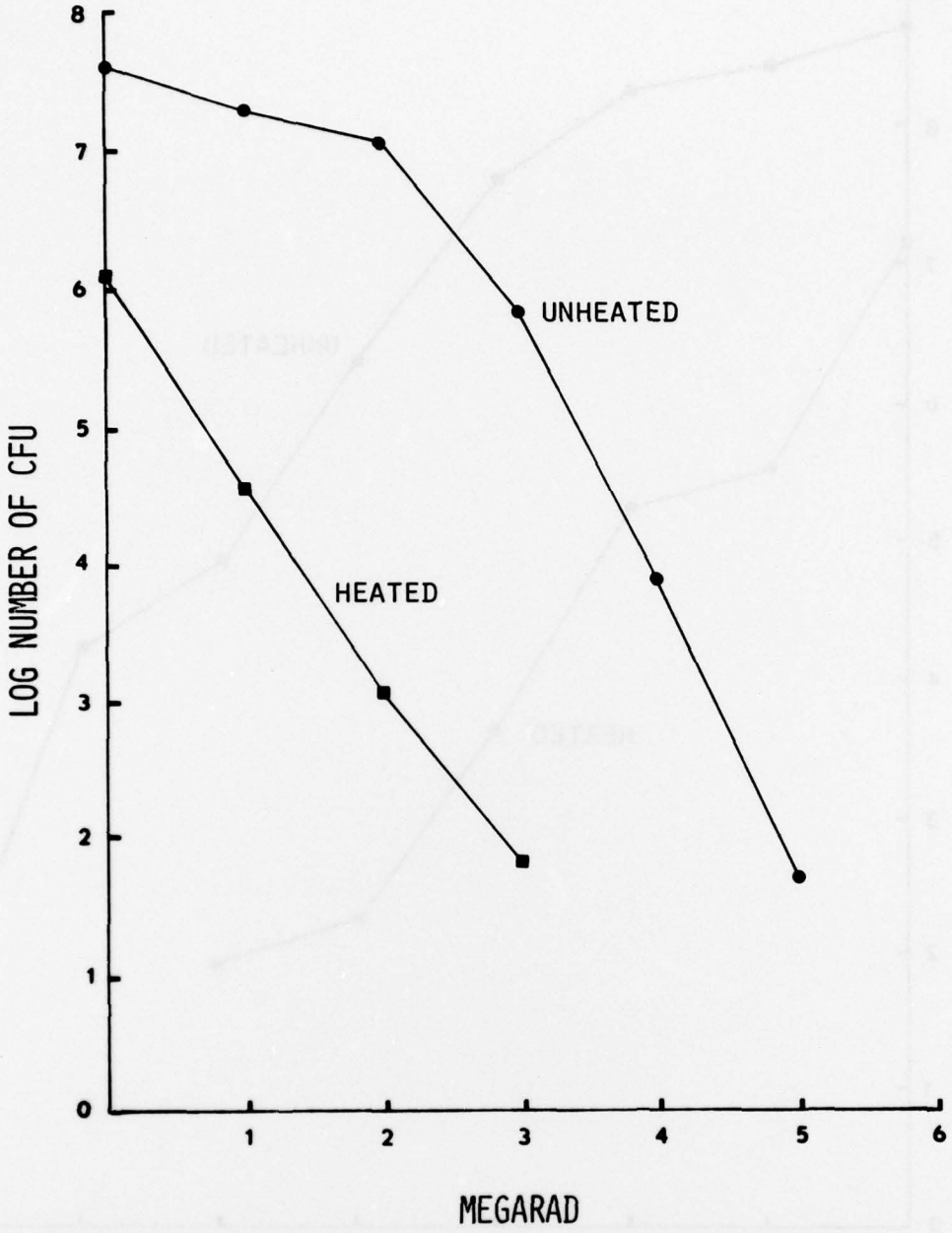

Figure 3. The effect of heating at 60°C for 8 minutes prior to freezing and irradiation on the death curve of Isolate 9.

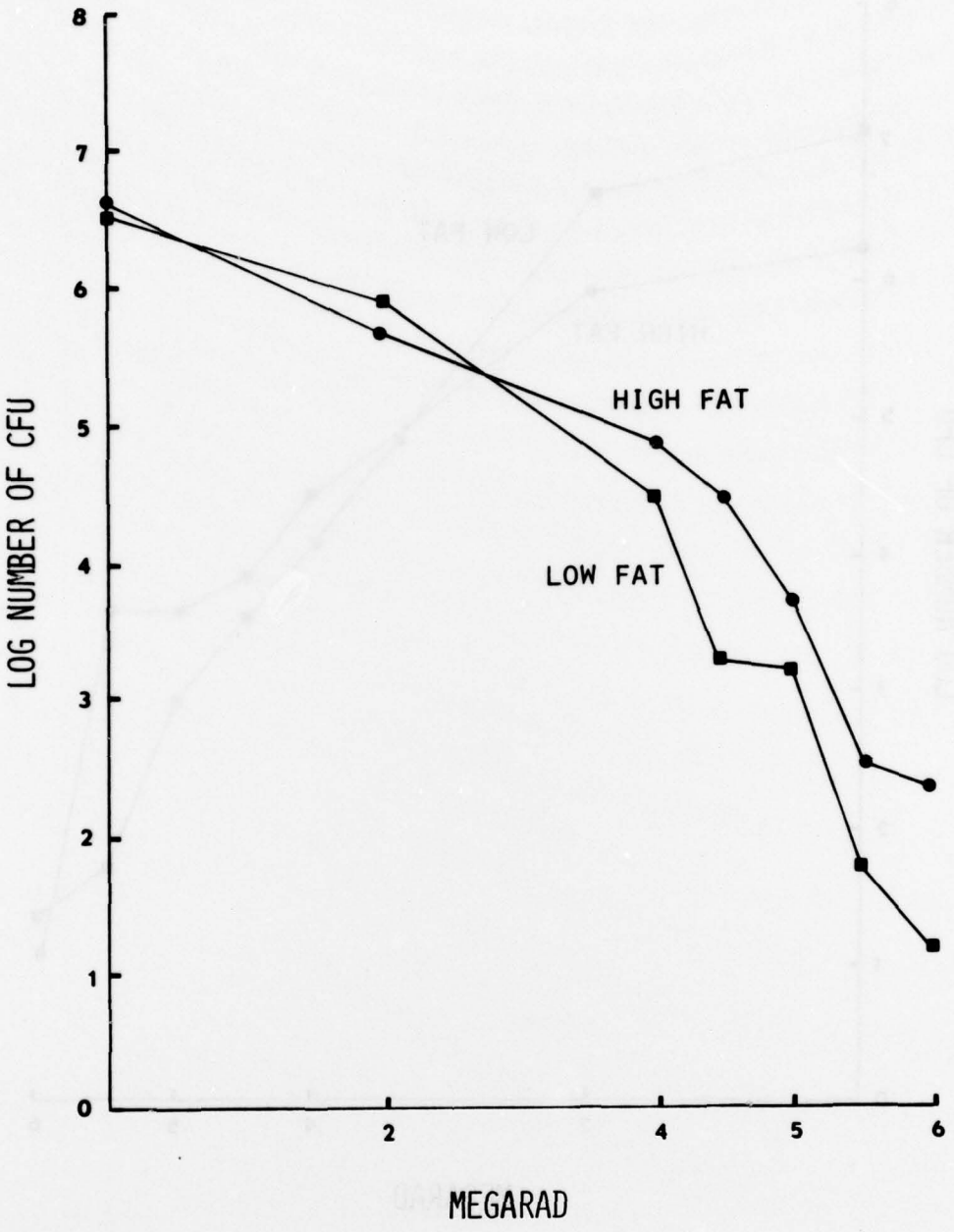

Figure 4. Comparative radiation resistance of Isolate 4 in low fat and in high fat ground beef.

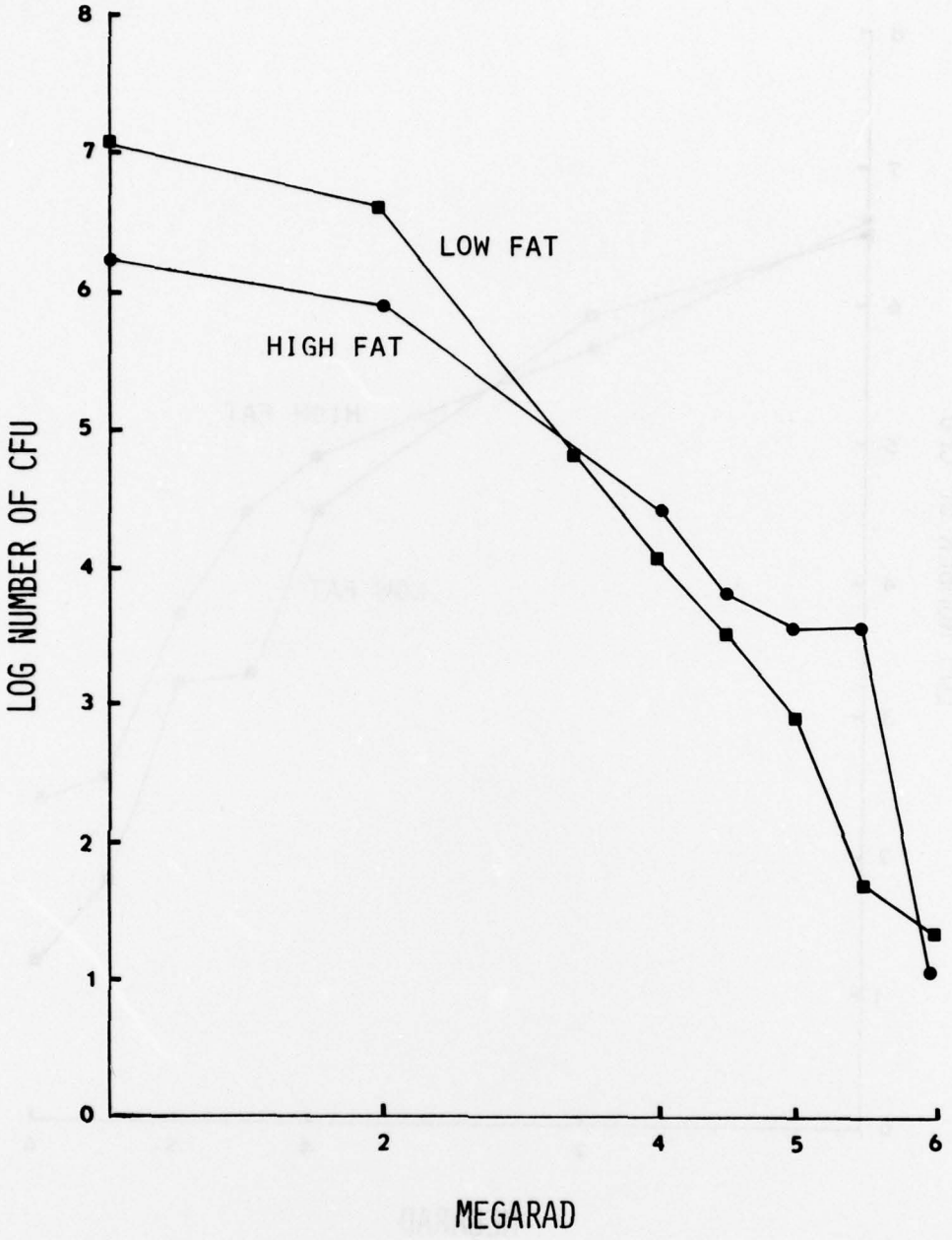

Figure 5. Comparative radiation resistance of Isolate 7 in low fat beef and in high fat beef.

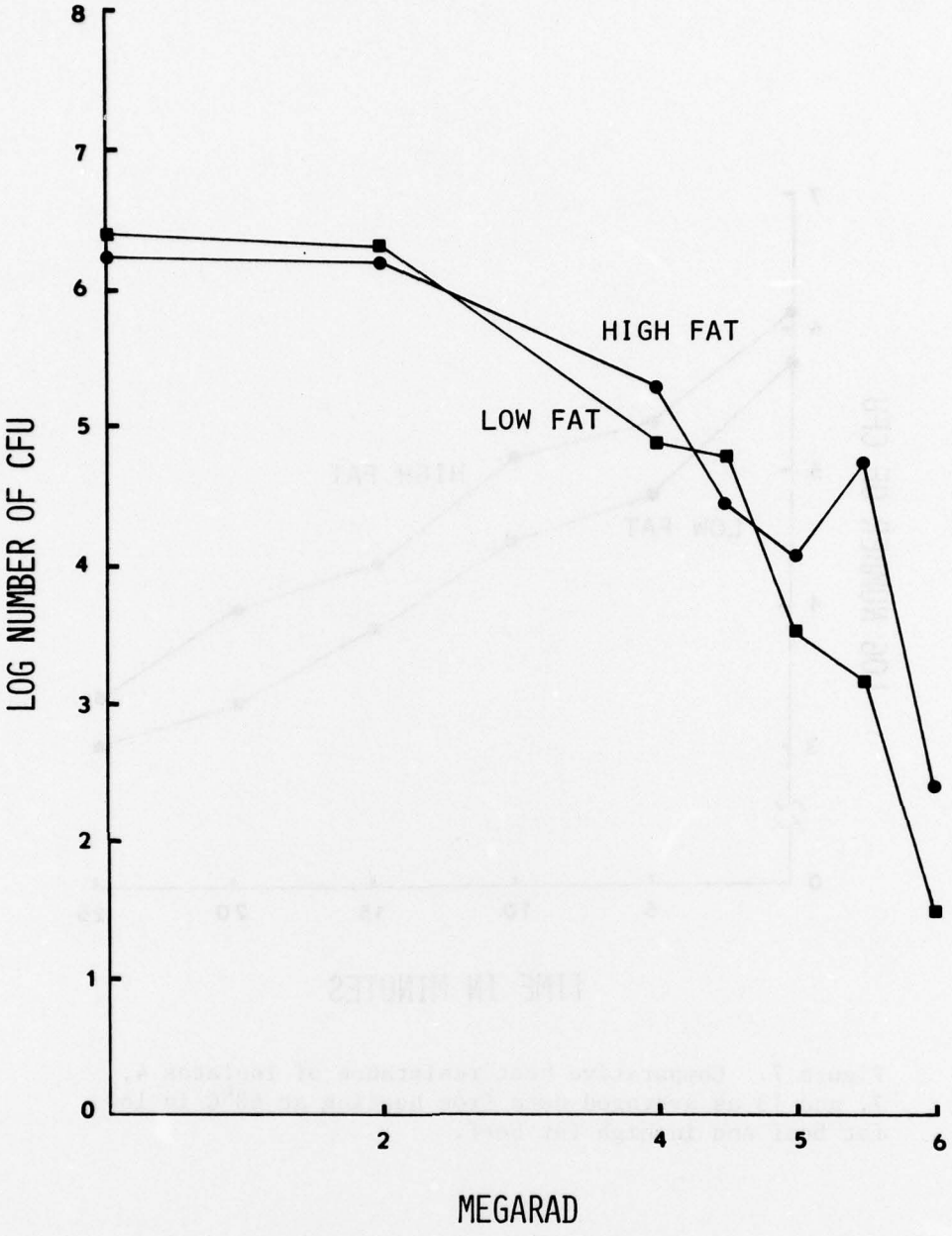

Figure 6. Comparative radiation resistance of Isolate 13 in low fat beef and in high fat beef.

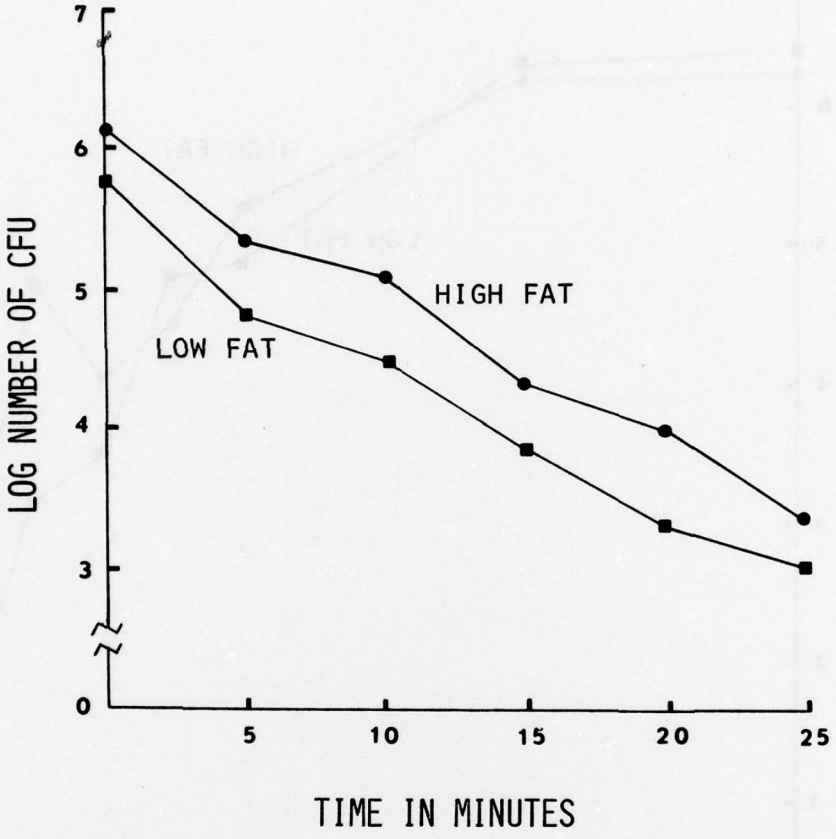

Figure 7. Comparative heat resistance of Isolates 4, 7, and 13 as averaged data from heating at 68°C in low fat beef and in high fat beef.

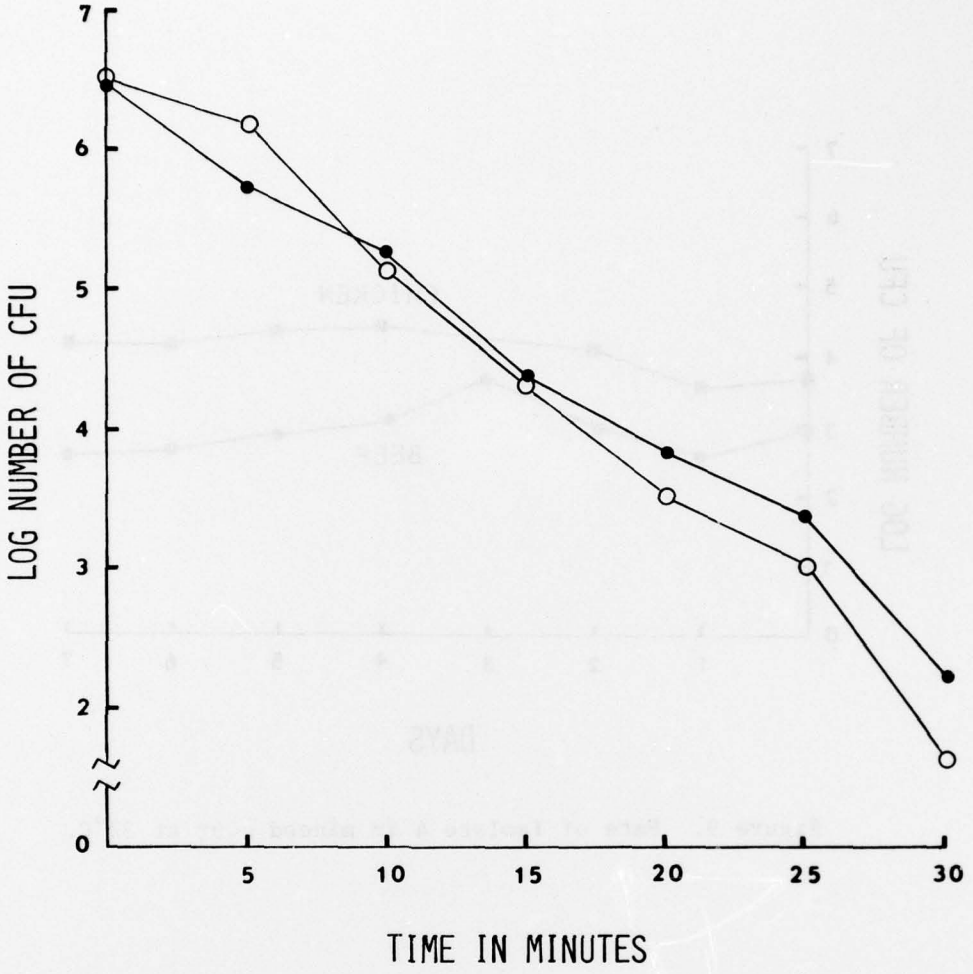

Figure 8. Heat resistance of Isolates 4 and 7 in chicken at 72°C. Open circles indicate Isolate 4; closed circles indicate Isolate 7.

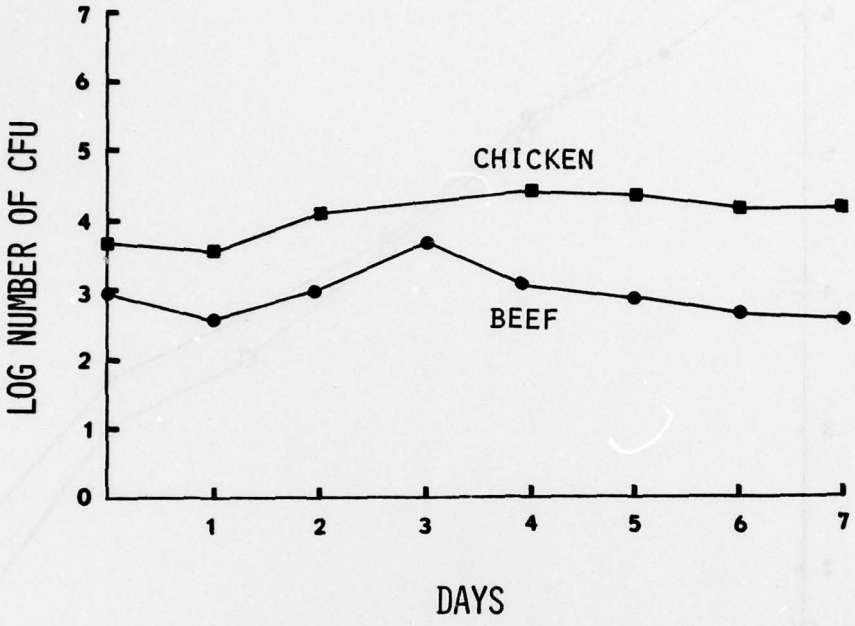

Figure 9. Fate of Isolate 4 in minced meat at 32°C.